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MECHANICAL PROPERTIES OF MAMMALIAN MYOCARDIUM

Influence of Muscle Length and Contraction Frequency

Lund 1972

From the Department of Pharmacology, University of Lund, Sweden

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- III NILSSON, E., Influence of muscle length on the mechanical parameters of myocardial contraction. Acta physiol. scand. 1972. In press.
- IV EDMAN, K. A. P. and E. NILSSON, The dynamics of the inotropic change produced by altered pacing of rabbit papillary muscle. Acta physiol. scand. 1969. 76. 236-247.
- V JÓHANSSON, M. and E. NILSSON, A note on the influence of asynchronous activation on myocardial contraction. Acta physiol. scand. 1972. In press.
- VI GENNSER, G. and E. NILSSON, The relation between the action potential and the active state in human fetal myocardium and its dependence on muscle length and contraction frequency. Acta physiol. scand. 1968. 73. 42-53.
- VII GENNSER, G. and E. NILSSON, Excitation and impulse conduction in the human fetal heart. Acta physiol. scand. 1970. 79. 305-320.

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1 INTRODUCTION

The present study concerns the effects of muscle length and contraction frequency on myocardial contraction in vitro. The analytical approach is based on the contraction model advanced by A. V. Hill (1938). Hill suggested that skeletal muscle could be represented by an active contractile element in series with an undamped elastic element. When considering the resting force of muscle, Hill (1951 b) introduced a second elastic element into the model, in parallel with the other two components. After stimulation, the contractile element is very rapidly brought into an active state. During this active state, the mechanical properties of the contractile element can be described by its force-velocity relation i.e. the velocity of shortening of the element as a function of force. This conceptual model could account for the fact that the capacity of the muscle to bear force is equally great (or nearly so) for a short time immediately after the latency period in a single twitch as it is during a tetanus. The model was also compatible with a number of other known properties of skeletal muscle, e.g., that the peak isometric force of a twitch is always lower than the force during a tetanus, although the contractile element is fully, or almost fully, activated during the initial phase of force development in a twitch (Ritchie 1954 a, b). On the basis of Hill's model it has been possible to derive methods for studying the time course of the active state in frog sartorius muscle (Jewell and Wilkie 1958, 1960) and also in single muscle fibres of the frog (Edman, Grieve and Nilsson 1966; Edman 1970; Edman and Kiessling 1971). It is now generally accepted that muscle contraction involves an interaction between two sets of filaments, the thick (A) and the thin (I) filaments. (A. F. Huxley and Niedergerke 1954; H. E. Huxley and Hanson 1954; H. E. Huxley 1969). According to the sliding-filament mechanism, the two sets of interdigitating filaments slide along each other during contraction. These filaments may be considered as the ultrastructural counterpart of Hill's contractile element. Depending on whether the ends of a muscle are fixed or freely movable, the muscle either produces force or shortens actively during contraction. At any sarcomere length, the isometric tetanic force of single skeletal muscle fibres is proportional to the area of overlap between the thick and the thin filaments (Edman 1966; Gordon, Huxley and Julian 1966). It has also been demonstrated that the unique relation between force and velocity of shortening exists on the single fibre level (Edman and Grieve 1966; Podolsky and Teichholz 1970; Julian 1971). The force-velocity relation has been considered of major interest in the

analysis of the molecular events in the contractile process (Huxley 1957).

The influence of muscle length on heart muscle contraction has been extensively studied since the early observation of Frank (1895) and Patterson, Piper and Starling (1914) that the stroke volume of isolated whole hearts increased with increasing diastolic volume. (For references, see Blinks and Koch-Weser (1963).) These investigations have shown that optimum active force in myocardium is reached at lengths where an appreciable resting force exists. In the last few years it has also been possible to compare active force production and sarcomere length in heart muscle. In contrast to the situation in skeletal muscle, it has been found that an appreciable resting force exists at lengths where the area of overlap between the thick and the thin filaments reaches maximum value (Spotnitz, Sonnenblick and Spiro 1966; Grimm and Whitehorn 1968; Grimm et al. 1970; Leyton, Spotnitz and Sonnenblick 1971).

The effects of contraction frequency on heart muscle contraction have also been investigated extensively since Bowditch (1871) and Woodbury (1914) demonstrated that changes of the stimulation interval altered the strength of the myocardial contraction (for references, see Koch-Weser and Blinks 1963). It has generally been found that at physiological temperature the isometric force of excised ventricular preparations increases with contraction frequency up to frequencies within the limits considered normal for the particular species (Trautwein and Dudel 1954 a, b; Koch-Weser 1963; Gennser, Jóhannsson and Nilsson 1972).

Abbot and Mommaerts (1959) demonstrated that it is possible to study the contractile process in myocardium with techniques similar to those previously applied to skeletal muscle. Subsequent works (Brady 1966; Edman, Grieve and Nilsson 1966; Sonnenblick 1967) revealed important differences between contraction in skeletal muscle and myocardium, notably the slow onset of the active state in heart compared with skeletal muscle. These studies also indicated that the mechanical analysis of myocardial contraction is complicated by the fact that heart muscle cannot normally be tetanized.

At the start of the present work, it was conceived that studies of the mechanical parameters of the myocardial contraction would open up new possibilities for analysing the excitation-contraction coupling process in heart muscle. The following questions were considered of particular interest. How is the force-velocity relation of the contractile element in heart muscle altered with time during a single contraction cycle? How does muscle length and contraction frequency affect the force-velocity relation? Can the concept of active state be applied to heart muscle in the same sense as used in skeletal muscle, i. e. to indicate the force-

bearing capacity of the contractile element at constant length? If so, how are the intensity and the time course of the active state affected by changes in muscle length and contraction frequency? Most of these studies were performed on papillary muscles of rabbit. In order to see to what extent the results obtained were applicable also to man, some measurements were made on human foetal papillary muscles obtained at midgestation. With the object of establishing the general development of foetal heart cells at this developmental age, an electrophysiological study of human foetal hearts was also undertaken.

2 MATERIAL

Rabbit heart. Papillary muscles were dissected from right ventricles of rabbits, weighing 1.0-1.2 kg. Preparation of papillary muscles is described in (I) and (II). Mounting of the preparation to the apparatus is shown in Fig. 1, insert.

Human foetal heart. Foetal hearts were obtained after legal interruptions of pregnancy at the department of Obstetrics and Gynaecology at the General Hospital, Malmö. Estimated age of the foetuses ranged from 17 to 24 weeks and their crown-heel length from 18 to 27 cm. Dissection and mounting of papillary muscles from foetal hearts were carried out essentially as described for rabbit papillary muscles. Hearts were opened by incisions in the anterior walls of atria and ventricles for electrophysiological recordings from the interior parts of the hearts, as described in (VII). In some cases, the hearts were perfused via the coronary system ad modum Langendorff during the electrophysiological recordings.

3 METHODS

Release experiments. The recording technique used in the release experiments (I - IV, VI) is described in detail in (I) and (II). Briefly, the muscle was connected via platinum loops to a force transducer (RCA 5734) and a straightened steel wire extending down from a lever (displacement transducer) as shown in Fig. 1. Three different levers were used. The equivalent mass of the levers, including the steel wire and the spring used for loading of the preparation, ranged from 70 to 150 mg. In order to prevent inertial oscillations of the lever, its movements were damped by means of a peg with a small disc on its lower end (B, Fig. 1). The disc was immersed in silicon oil, viscosity 1000-12500 centistokes. /Not 200-1250 centistokes as erroneously stated in (I), page 207./ The natural frequency of the length recording system under the experimental conditions used is described in (III), Appendix 1. Resting length of the preparation was set by means of a stop screw (D, Fig. 1). The lever was locked against this stop screw by means of a releasable catch.

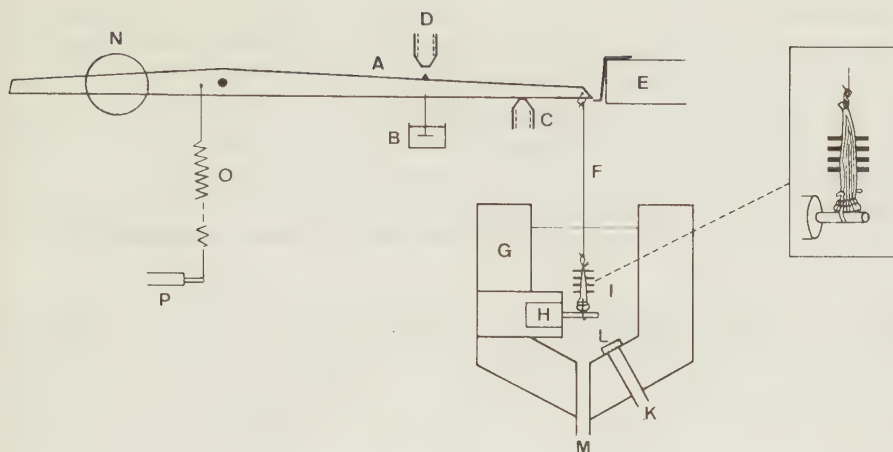


Fig. 1

Schematic illustration of arrangement used for release experiments.

A, Isotonic lever; B, damping dashpot containing silicon fluid; C, stop screw (used in stiffness determinations only); D, stop screw for setting of resting muscle length; E, Releasable catch; F, Straightened steel wire; G, Thermostated bath; H, RCA 5734 force transducer; I, Papillary muscle and stimulating electrodes; K, Inlet for gas; L, Filter; M, Suction drain; N, Photocell; O, Coil or rubber spring; P, Strain gauge transducer. Insert figure shows mounting of papillary muscle on a larger scale. Platinum loops are tied to both ends of the papillary muscle with silk threads. The upper platinum loop is connected to the steel wire; the lower, to the RCA transducer.

The catch could be electromagnetically withdrawn at any pre-set time after stimulation and development of isometric tension to enable isotonic shortening of the preparation. Loading of the preparation was achieved by hanging weights on a coil or rubber spring attached to the lever on the side of the fulcrum, opposite the muscle. In later experiments (II, III) the lower end of the spring was connected to a strain gauge transducer which recorded the tension in the spring.

Experiments on asynchronous activation. To make it possible to study the effects of asynchronous activation on the myocardial contraction, a preparation was used consisting of two papillary muscles mechanically arranged in series. The interval between the stimulation of the two muscles and the order of stimulation could be varied. The two papillary

muscles were placed horizontally opposite each other, as described in detail in (V). One end of each muscle was connected to opposite faces of the same vertical lever via platinum loops, the other end of each muscle was fixed to separate force transducers (RCA 5734). The position of the lever (and hence the length changes of each muscle), was recorded by means of a capacitance transducer. When the lever was fixed the isometric force of each muscle could be measured. When the lever was freely movable, the force of the muscles was measured when they contracted in series.

Micro-electrode recordings. Conventional electrophysiological micro-electrode technique, as described in (VI) and (VII), was used.

4 RESULTS

Experiments on rabbit papillary muscles

Mechanical parameters of myocardial contraction (I and II)

The analytical approach of the present studies is described in (I). At low resting force the muscle is considered to function as an active contractile element in series with an undamped elastic element. The force-velocity relation of the contractile component was determined at a given instant during contraction by releasing the muscle to shorten against various loads. The shortening velocity was measured after the series elastic element had readjusted itself to the load. Force-velocity curves obtained at constant contractile element length at various times during the contraction cycle had a hyperbolic shape and could be fitted by Hill's equation using the same values for the constants a and b . This means that extrapolated values of P_0 and $V_{\max}$ change in parallel throughout an isometric twitch. As discrepant results had been obtained by other workers (Brady 1965; Noble, Bowen and Hefner 1969) the question of the shape of the force-velocity curve was taken up for further investigation (II). It was demonstrated that the outcome of the force-velocity analyses was greatly dependent on the degree of damping of the lever during the release procedure. When the lever movement was critically damped, the curves obtained exhibited a true hyperbolic shape. Virtually the same constants a and b in Hill's equation were obtained when the analysis was carried out during the rising and the falling phases of the isometric contraction. On the other hand, quick releases without critical damping of the lever movements were found to have a depressant effect on the myocardial contractility resulting in a distortion of the force-velocity curve at intermediate and low loads. This distortion of the curve was more pronounced when the analysis was carried out during the rising phase of the contraction than during relaxation. Because of the parallel shift of the force-velocity relation throughout a contraction cycle, the shortening velocity of the contractile element at a low load, measured at a given length of this element, can be used as a true index of the intensity of the active state. The active state in papillary muscle rises very slowly, $1/3$ to $1/2$ of the rising phase of the isometric twitch being necessary for attainment of full activity. The duration of the active state, determined from the isotonic myograms after different degrees of

shortening of the contractile element, was found to decrease with decreasing contractile element length. It was concluded that the time course of the active state is not definitely programmed at the start of a contraction, but is affected by changes in length of the contractile element during the contraction cycle. The influence of contractile element length per se on the active state, as distinct from the effects of active shortening, is considered in the following study (III).

The stiffness of the series elastic element was found to increase rectilinearly with force. The stiffness was found to be the same at a given level of force on the rising and the falling phases of the twitch, which suggested that the intensity of the active state did not affect the series elastic stiffness.

Influence of muscle length (III)

In (III) the dynamic properties of papillary muscles were analysed according to the two muscle analogues most commonly used in investigations of mechanical properties of skeletal muscle and myocardium. Each of these models is composed of a contractile element, a series elastic element, and a parallel elastic element. In the 'Maxwell model' (here denoted model I), the parallel elastic element is located in parallel with both the series elastic and the contractile elements. In the 'Voigt model' (here denoted model II), the parallel elastic element is situated in parallel with the contractile element only. In the present analysis, it is assumed that the contractile element in heart muscle does not bear any resting force. This is based on the following considerations. 1. Brady (1968) summarized pertinent biochemical data on myocardial elastic tissue and reached the conclusion that cardiac resting force can be attributed to extracellular collagen and to sarcolemma. 2. The contractile activity can be drastically changed by addition of Co^{2+} to the perfusing solution and by changes in temperature, without significant changes in resting stiffness or in resting length-tension curve (Mattiazzi and Nilsson, unpublished). It is also implicit in the analysis that the elastic elements can bear force in one direction only, i. e. they go slack when compressed.

Muscle stiffness was found to increase rectilinearly with force in both resting muscles and in active muscles contracting at lengths where resting force is low. At any force level, the stiffness of a resting preparation exceeded that of the same preparation during activity. This was shown to be incompatible with the arrangement of the parallel elasticity in model II. The static stiffness of resting muscle, defined as the slope of the resting length-tension curve, was less than the

dynamic stiffness, indicating viscous properties of resting myocardium. Muscle viscosity was also evidenced during releases of resting preparations from high to low force levels. During such releases, the length of the preparation changed in two phases. After an initial rapid change, which may be ascribed to an undamped elastic element, a very slow decrease in muscle length occurred (creep). The length change during such a creep, attributable to readjustment of a viscous element, was too slow to significantly affect velocity measurements in active preparations. Hence, the viscous element could be ignored.

Force-velocity curves of papillary muscles at different resting lengths were obtained after releases from peak twitch tension. The corresponding force-velocity curves of the contractile element alone were calculated from experimental data assuming that the parallel elasticity of the preparation was located according to muscle model I. The extrapolated $V_{\max}$ of the contractile element increased with muscle length up to lengths where active force reached a maximum.

The time course of the active state was determined at different contractile element lengths. With increasing length, the duration of the active state was prolonged. This effect was mainly due to a later onset of the decay of the activity. No significant influence of length on the rising phase of the active state was observed.

Influence of contraction frequency (IV)

The influence of changes in contraction frequency on the mechanical parameters of the myocardial contraction was studied. Force-velocity curves were obtained at different contraction frequencies as described in (I) and (II). By releasing the preparation from the same force level before and after a change in contraction frequency, it was possible to refer all velocity measurements at two different frequencies to the same length of the contractile element. Changes in contraction frequency produced a shift of the force-velocity curve without markedly affecting the shape of the curve, suggesting that $V_{\max}$ and P_0 were altered to approximately the same extent. Intensification of active state in response to an increase in stimulation frequency was associated with a steeper rise of the activity and an earlier onset of the decay phase. Frequency-induced inotropic changes did not affect the relation between stiffness and force of the series elastic element.

Asynchrony of contraction (V)

In order to study the effect of asynchronous activation of myocardial contraction a preparation was used consisting of two papillary muscles mechanically arranged in series. This implied that the force of the two muscles were equal, and that at any moment during contraction the muscle with the greatest force-producing capacity would stretch the other muscle. At 37°C, an interval of 25 or 50 msec between stimulation of the two muscles increased the movements of the muscles during contraction, reduced the peak active force of the contraction, and prolonged its time course. These effects of contractile asynchrony increased with increasing frequency within the range 30-150 contractions per min.

Experiments on human foetal heart preparations

Influence of muscle length and contraction frequency on the kinetics of active state and on action potential duration (VI)

The resting and active length-force curves of human foetal papillary muscles were found to be similar to those obtained from papillary muscles of adult rabbits. Resting force increased with length in an exponential manner. Optimum active force was reached at a length 1.3 times l_0 (l_0 = the length of just detectable resting force). The relation between contraction frequency and active force was studied at 30°C. This temperature was chosen because at 30°C the contraction of papillary muscles is slow enough to allow sufficient time resolution of active state measurements, see below. The active force was found to be optimum at a contraction frequency of 84 beats per min.

The time course of the active state was measured as described in (I) and (IV) at a constant length of the contractile element. An increase in contraction frequency from 36 to 72 contractions per min intensified the active state and decreased the time needed for attainment of 90 % of maximum activity and also the time from stimulus to 50 % decay of the active state. These changes of the time course of the active state were accompanied by a shortening of the duration of the action potential, determined at the level of 50 % maximum amplitude. As previously described for rabbit papillary muscles (II), the duration of the active state, measured on the same myograms at successively shorter lengths, decreased with decreasing contractile element length. Changes in muscle length did not affect the duration of the action potential.

Electrophysiology of human foetal heart (VII)

The aim of this report was to study the electrophysiological characteristics of foetal heart cells at midgestation. The shape of action potentials in various regions of the heart is described. Diastolic depolarizations and subthreshold oscillations indicating latent pacemaker activity were found in atrial but not ventricle cells. Resting membrane potential was -81.4 ± 1.6 mV (mean $\pm$ SEM) in ventricular cells at 4 mM external potassium concentration, $/K^+/e$, and increased 51.2 mV per tenfold increase in $/K^+/e$, indicating a high potassium conductance in resting cells. At low $/K^+/e$ (0.5 mM) spontaneous action potentials appeared in ventricle cells. The conduction velocity was 0.4–0.7 m/sec in both atrium and ventricle, approximately 0.01 m/sec in the AV-node. Conduction velocity decreased at high contraction frequencies in all regions of the heart, in the atrium it also decreased at very low frequencies. It is concluded that, apart from a more widespread pacemaker potentiality in atrial cells, the electrophysiological characteristics of human foetal heart cells at midgestation are similar to those of adult cells from various mammalian species including man.

Consideration of methods

The aim of the present reports was to investigate the influence of changes in muscle length and contraction frequency on the mechanical parameters of myocardial contraction. In particular, it was considered of importance to study how the dynamic properties of the contractile element, i.e. the force-velocity relation and the time course of active state, are affected by these two interventions. Force-velocity and active state analyses are based on measurements of the shortening velocity of papillary muscles in releases during isometric twitches. The velocity determinations are carried out at times when the series elastic element has adjusted its length to the isotonic load of the preparation. Before discussing the results obtained, it is appropriate to consider two arguments raised against this kind of analytical approach. It has been suggested (see Brady 1968; Sonnenblick and Stam 1969) that the rapid length change of a myocardial preparation after a release induces 'uncoupling', i.e. deactivation of the contractile element. In (II) it is shown that underdamped releases do cause such a deactivation of the contractile element, the more so, the later in the contractile cycle the release is performed. However, this deactivating effect of the release does not occur if the release is damped to an extent that prevents mechanical oscillations from occurring during the release. Throughout the course of the present study, great care was taken to adjust the degree of damping so as to reduce the deactivating effect of the release procedure to the point where it was negligible.

A second objection to the analytical technique employed in the present study concerns the use of muscle analogues that have no viscous component. It has been claimed that elasticity of muscle cannot be adequately represented by undamped elastic elements (Bleichert and Reichel 1962, 1968; Reichel and Bleichert 1962; Rumberger 1970; Rumberger and Schwartz 1969). According to these authors elastic properties of muscle are best described by a visco-elastic unit common to both resting and active muscle. The term 'Aktivierung' or 'Kontraktionsfähigkeit' are used to denote the force-producing capacity during the contractile process. 'Kontraktionsfähigkeit' is measured by taking the difference between the force redeveloped by an active preparation after a release (a few per cent of muscle length) and the force redeveloped after a release of the resting

preparation from the same initial force level. This method has been critically discussed by Blinks and Koch-Weser (1963) and by Brady (1968). As pointed out by Brady (1968), one problem behind the concept of 'Aktivierung' is the assumption that the stiffness of the contractile element is the same at rest and during activity. It may be added that the outcome of such an analysis is strictly dependent on the amount of release and on the time after the release when recovery forces in active and resting muscle are determined.

Many studies support the view that resting heart muscle behaves as if it contained highly damped elastic elements in addition to the undamped elastic elements of the three component muscle models. The occurrence of creep and stress relaxation (see Blinks and Koch-Weser 1963; Little and Wead 1971; Wead and Little 1971), redevelopment of force after releases of resting preparations (Rumberger and Schwartz 1969), and the fact that dynamic stiffness of resting myocardium exceeds its static stiffness (Lundin 1944, and present study (III)), are all indications of viscous properties of heart muscle. However, length changes attributable to muscle viscosity after a damped release are of small magnitude and have too slow a time course to affect velocity measurements during releases of active preparations (III). It can therefore be concluded that the determinations of shortening velocity of the contractile element used in the present series of investigations, are not seriously affected by viscous properties of the myocardium.

In their most generalized forms, the equations describing the dynamic properties of the two models of muscular contraction considered in the present study, can be shown to be interconvertible, i. e. results explained by one model can also be described by the other (Buchtal, Kaiser and Rosenfalck 1951; Fung 1971). But in the present analysis two assumptions were made, 1. that the contractile element is freely extensible at rest; 2. that the elastic elements go slack and do not exert force when compressed. On this basis, the models were considered as separate entities. The results of stiffness determinations (III) were found compatible only with the model in which the parallel elastic element is arranged in parallel with both the contractile element and the series elastic element (muscle model I or 'Maxwell model').

Force-velocity relationship

The finding that $V_{\max}$ and P_0 of the instantaneous force-velocity curve in myocardium vary to the same extent with time during a contraction

cycle (I and II) is of relevance to understand myocardial mechanics as it enables active state curves measured in terms of shortening velocity to be transformed into 'force active state curves' (see below). The same type of change of the myocardial force-velocity curve (a proportional increase in $V_{\max}$ and P_0) is also induced by inotropic interventions, such as changes of contraction rate (IV), alterations of external calcium concentration, and ouabain and adrenalin (Edman and Mattiazzi, unpublished). On the other hand, temperature changes affect the force-velocity curve asymmetrically. An increase in temperature raises $V_{\max}$ relative to P_0 (Mattiazzi and Nilsson, unpublished). Some caution is required in the quantitative evaluation of changes in the instantaneous relation between force and velocity in myocardium as the intrinsic resistance to shortening in heart cells is unknown (II and IV). However, as pointed out in (IV), although an internal load would tend to reduce shortening velocities measured at low external loads, it cannot explain the fact that $V_{\max}$ and P_0 are altered to the same extent by inotropic interventions. Therefore, it is of interest to consider the results of the present study in the light of current views concerning activation of contraction. In both skeletal muscle and myocardium, calcium is considered to function as activator of the contractile process by combining with the troponin-tropomyosin complex, which is distributed along the thin filaments (Ebashi and Endo 1968; Katz 1970). The number of thin filament sites that can interact with myosin is assumed to depend on the number of troponin molecules that have formed a complex with calcium. This concept explains why alterations in calcium activity in the myofibrillar space change the force-producing capacity of the contractile element. But, as discussed in (IV), it does not explain how alterations in $V_{\max}$ of the contractile element can occur if crossbridges between the thin and the thick filaments are assumed to function in an all or none manner (cf. also Katz, Goodhart and Goodhart 1971). Recently, the force-velocity relation has been studied in single skeletal muscle fibres of the frog (Podolsky and Teichholz 1970; Julian 1971) and in glycerinated rabbit psoas muscle fibres (Wise, Rondinone and Briggs 1971). In these investigations, contradictory results have been obtained concerning the influence of changes in calcium concentration on $V_{\max}$.

Several recent studies make it reasonable to believe that present views concerning the role of calcium in contraction are inadequate. Two reports suggest different mechanisms which can possibly explain variations in $V_{\max}$ based on alterations in the rate of crossbridge movement. X-ray studies have shown that electrical activation of frog sartorius muscle produces movement of myosin crossbridges even in the complete absence of overlap between myosin and actin filaments (H. E. Huxley 1971) and this suggests a direct effect of calcium on the crossbridges, apart from its action on the troponin site. A new theory of muscle contraction (A. F.

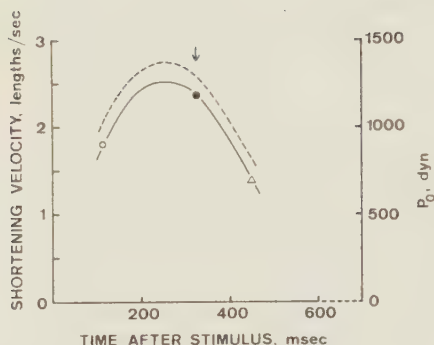
Huxley and Simmons 1971) proposes that crossbridge movement takes place, not in a single step but in two or perhaps more steps. According to this theory, the overall response at the crossbridge level might be gradable.

Active state and excitation-contraction coupling

In the present reports (I, III, IV, VI), the intensity of the active state was experimentally determined in terms of shortening velocity of the contractile element at a given length of this element. In this way, the influence of changes in contractile element length occurring during an ordinary isometric contraction were avoided. The active state data of the present study were collected when the contractile element passed the length which it attains at the peak of the isometric twitch. As the peak force represents zero velocity of the contractile element, this force also indicates the actual capacity of the contractile element to produce tension at this instant. With this value as a reference, the experimentally obtained 'velocity-active state' curves can be transformed into corresponding 'force-active state' curves as illustrated in Fig. 2. This transformation is based on the finding, previously discussed, that $V_{\max}$ and P_0 of the myocardial force-velocity curve are altered to the same extent with time throughout a contraction cycle.

Active state curves, referring to the length of the contractile element existing at peak isometric force, demonstrate that the active state has a slow onset and lacks a distinct plateau phase. The intensity of the active state is determined both by contractile element length and degree of activation of the contractile element (see below). From the results of the present study (I, II, III) it may be concluded that the capacity of the contractile element to bear force only slightly exceeds the externally manifested isometric tension during the rising phase of a twitch (cf. Fig. 2 in (II)). The slow onset of the active state observed in the present study agrees with previous findings of Brady (1966) Sonnenblick (1967). The former author showed that quick stretches applied during the first half of the rising phase of an isometric twitch produced responses nearly identical to those seen when the same stretches were applied before the stimulus. Furthermore, the shortening velocity during afterloaded contractions with light loads increases for an appreciable time after onset of the isotonic shortening phase. This has also been taken as an indication that the intensity of the active state increases during most of the rising phase of an isometric twitch

A



B

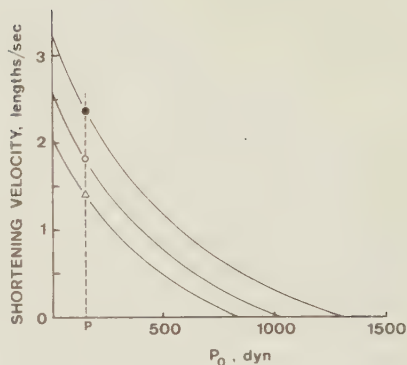


Fig. 2

Schematic illustration of method used for determining the time course of the active state terms of force

Fig. 2A shows (full line) the shortening velocity of the contractile element at load P (see Fig. 2B) tested by means of release technique at different times after stimulation. Dotted line denotes the time course of the muscle's capacity to produce tension (P_0). All velocity data refer to the length of the contractile element existing at peak twitch tension. Measurements made at three different times during the contraction cycle are indicated. Instantaneous force-velocity curves existing at these moments are illustrated in Fig. 2B. Dotted line of Fig. 2B shows the isotonic load used in velocity measurements of Fig. 2A. As the shape of the myocardial force-velocity curves remains constant throughout a contraction, Hill's equation can be written $P_{0t} = V_t \cdot K + P$, where P_{0t} is the intensity of the active state at time t , V_t the isotonic shortening velocity against load P at time t and $K = \frac{P + a}{b}$. At peak twitch tension (indicated by arrow in Fig. 2A) P_0 equals the isometric force. Hence, the constant K can be calculated, and therefore also the P_0 -value corresponding to each isotonic velocity determination of Fig. 2A.

(Brady 1966; present report (II)). Some recent experiments have been interpreted to show that the intensity of the active state rises more steeply and that the myocardial force-velocity relation is independent of time during the major part of tension development of an isometric twitch (Brutsaert, Claes and Sonnenblick 1971a). These conclusions contradict earlier findings discussed above, including the results of the present study.

The slow onset of the active state in heart muscle is in contrast to the situation in skeletal muscle where several studies indicate a very rapid onset. The resistance to quick stretch rises immediately after the latency period and remains at a high level throughout the plateau phase of the active state (Hill 1949). The active state, defined as the capacity to bear tension, rises from 25 to 65 % of its maximum values within 3-4 msec as determined in single muscle fibres (Edman 1970). Furthermore, the shortening velocity of lightly loaded skeletal muscle reaches its maximum value 7-8 msec after the end of latency period (Hill 1951 a). These differences between the time course of the active state in myocardium and in skeletal muscle are of major interest in the study of excitation-contraction coupling.

The intensity of the active state at a given instant is not predetermined at the start of a contraction. It is affected by the history of the contractile element before the active state measurement is made. Passive stretch (Brady 1966), active shortening (Brady 1966, 1968; Edman and Nilsson 1971), and undamped releases (present study (II)) reduce the intensity of the active state, the more so, the later in the time course of the active state that the displacement occurs. Brady (1968) suggested that it might be useful to define two types of myocardial contractility: static and dynamic. Both should be measured in terms of force: the former at constant contractile element length, the latter when the contractile element is moving at constant speed. In view of the technical difficulties inherent in this approach, it seems preferable, to the present author, to use the term active state to refer to the maximum force-producing capacity of the contractile element at a given length, irrespective of prehistory of the muscle. In addition to the depressant effects of displacement, evidence has been presented to show that active shortening of the contractile element tends to prolong the cardiac action potential, thereby increasing the intensity of subsequent beats (Kaufmann et al. 1971).

The force that the contractile element can bear without lengthening or shortening may be taken as an index of the amount of calcium bound to the contractile proteins (Sandow, Taylor and Preisler 1965; Edman, Grieve and Nilsson 1966; Taylor 1969; Sandow 1970; Edman and Kiessling 1971). This view does not depend on whether the activity of the crossbridges is regulated in an 'on-off' or in a graded manner. Active state determinations, as performed in the present study, can therefore be used to disclose relevant information about the kinetics of the calcium release and inactivation processes. According to current concepts concerning excitation-contraction coupling in mammalian myocardium, the time course of calcium release from storing sites is in some way controlled by the action potential. The amount of calcium

bound to these intracellular stores before excitation is a complex function of calcium fluxes during preceding action potentials and diastolic intervals (Langer 1968; Little and Sleator 1969; Wood, Heppner and Weidmann 1969; Beeler and Reuter 1970; Gibbons and Fozzard 1971; Manring and Hollander 1971). In view of this, the slow onset of the myocardial active state is of interest, as it suggests that the release of calcium into the myofibrillar space exceeds the rate of calcium inactivation during the major portion of tension development.

Influence of muscle length and contraction frequency on kinetics of active state

In the present study, it was demonstrated that $V_{\max}$ of the contractile element increases with muscle length up to lengths where active force is maximum. This is in contrast to conclusions reached in an early study (Sonnenblick 1962), but in apparent agreement with results recently presented by Brutsaert, Claes and Sonnenblick (1971 b). In the latter study, shortening velocities were measured during afterloaded contractions with the load being corrected for parallel elasticity. It should be pointed out that, with this technique, velocity determinations at different lengths of the contractile element refer to various times during the contraction cycle. The interpretation of these results therefore depends upon presumptions concerning the time course of the active state (cf. above, Active state and excitation-contraction coupling).

In (III), it is also shown that, with increasing muscle length, the duration of the active state is prolonged. It is of interest that an increase in active state duration, in response to elongation in single skeletal muscle fibres, was tentatively attributed to a prolongation of release of activator calcium (Edman and Kiessling 1971). A similar interpretation may also apply to the corresponding phenomenon in myocardium.

Ultrastructural studies (Spotnitz, Sonnenblick and Spiro 1966; Grimm and Whitehorn 1968; Grimm *et al.* 1970) have shown that the average sarcomere length in heart cells increases with muscle elongation over the ascending portion of the length-active force curve. A reduction of the hindrance to crossbridge movement caused by double overlap of the thick filaments is generally taken as explanation for the observed enhancement of peak twitch tension. The increasing duration of the active state with muscle length, can be considered to be another mechanism independent of myofibrillar overlap tending to enhance the isometric force. The reduction of $V_{\max}$ with decreasing muscle length observed in the present study accords

with the idea of an increased internal resistance to shortening (Gordon, Huxley and Julian 1966). A reduced efficiency of the excitation-contraction coupling might also contribute to this effect by diminishing both P_0 and $V_{\max}$. Evidence showing that the degree of activity is reduced in skeletal muscle at very short sarcomere lengths has been presented by Taylor and Rüdel (1970).

Increases in contraction frequency affected P_0 and $V_{\max}$ of the force-velocity curve to the same extent, increased the intensity of the active state, reduced the time for attainment of 90 % of maximum activity and also the time to 50 % decay level. In order to get a sufficient time resolution of the mechanical measurements, the present studies (IV and VI) were performed at temperatures of 26-30°C, where the mechanical response is slower than at 37°C. The low temperature made it impracticable to study the contractile events at contraction frequencies higher than 70-90 beats per min. There are reasons for believing that at 37°C and at higher frequencies (Trautwein and Dudel 1954 a, b; Koch-Weser 1963; Gennser, Jóhannsson and Nilsson 1972) the duration of the active state shortens with increasing contraction frequency. The outcome of the active state analysis can be interpreted to show that, with increasing frequency both the rate of calcium release into the myofibrillar space and its inactivation are enhanced, whereas the duration of the calcium release is abbreviated.

Function of intact heart. Dependence on:

Muscle fibre length

Ventricular function curves (Sarnoff 1955) are often used to describe the relation between stroke volume or stroke work and end diastolic pressure (Sarnoff and Mitchell 1961). The increase in mechanical performance with end diastolic pressure is ascribed to changes in end diastolic volume, whereas inotropic effects are characterized by an altered stroke volume at constant end diastolic pressure. Recently, diastolic pressure has been shown to rise steeply with sarcomere length. It has also been shown that in a normal heart, even at very high end diastolic pressure, average sarcomere length only slightly exceeds the optimum limit for tension development (Spotnitz, Sonnenblick and Spiro 1966; Sonnenblick *et al.* 1967; Grimm *et al.* 1970; Leyton, Spotnitz and Sonnenblick 1971). A quantitative evaluation of ventricular function curves in terms of cellular events is complicated for the following reasons.

1) End diastolic pressure of a ventricle is a complex function of the diastolic volume, the geometry of the ventricular cavity, and the stiffness of the chamber walls; pressure tending to increase exponentially with volume (Noble et al. 1969; Diamond et al. 1971). Furthermore, the relation between ventricular volume and myocardial fibre length, or sarcomere length, is complex too and depends on the geometry of the ventricle. (Assuming a spherical shape, the volume is a cubic function of circumference.)

2) For a given stroke work, tension production and active shortening at the myofibril or the sarcomere level is dependent on end diastolic volume. (For an extensive discussion of this problem see, e.g., Blinks and Koch-Weser (1963).) In order to reach a given intraventricular systolic pressure, the fibres have to exert greater tension at greater ventricular dimensions according to the law of La Place. On the other hand, the greater the end diastolic volume of the heart, the less will the fibres have to shorten in order to produce a given stroke volume at constant aortic pressure. This is because the rate of volume change of a sphere increases as the square of its radius. In this connexion, two factors considered in the present study are of interest, as they will tend to prolong the duration of the contraction and thus raise the effectiveness of the heart beat when the ventricular volume increases. a) The increased duration of the active state with increasing fibre length will prolong the duration of the mechanical systole. A prolongation of the systolic contraction induced by increasing end diastolic volume has also been demonstrated in the intact dog heart (Taylor et al. 1967). b) The amount of fibre shortening required for a given stroke volume decreases with increasing end diastolic volume (cf. above). This might be expected to reduce the degree of deactivation of the myocardial fibres caused by active shortening.

Many studies have been performed with the object of defining an index of the inotropic state of the myocardium which would be independent of myocardial fibre length (end diastolic volume) and afterload (aortic pressure). The rate of rise of intraventricular pressure $(dP/dt)_{\max}$ has been suggested as such an index (Wallace, Skinner and Mitchell 1963; Schaper, Lewi and Jageneau 1964; Furnival, Linden and Snow 1970; Morgenstern et al. 1970) even though most of these studies indicate that $(dP/dt)_{\max}$ is affected to some extent by changes in end diastolic volume. In recent years, the possibility of calculating the shortening velocity of the contractile element in the intact heart has been explored (Fry, Griggs and Greenfield 1964; Levine and Britman 1964). The extrapolated $V_{\max}$ of force-velocity curves, derived from isovolumetric pressure recordings, is taken as an index of the contractility of the myocardium (e.g., Sonnenblick, Parmley and Urschel 1969; Falsetti et al. 1971).

It should be noted that results obtained in this way are affected not only by the non-spherical geometry of the ventricle (Fry, Griggs and Greenfield 1964; Lewi, Schaper and Jageneau 1971); the asynchronous contraction of the ventricle (Rushmer 1956 a, b; Hawthorne 1961) can also significantly influence the outcome of such analyses as are discussed in the present report (V) (cf. below).

Contraction frequency

The influence of contraction frequency on myocardial contraction is more obvious on excised myocardial preparations than on whole hearts. At physiological temperature, both the isometric force and the rate of force development of **isolated** preparations, such as papillary muscles, increases with frequency up to rates within the normal physiological level (Trautwein and Dudel 1954 a, b; Koch-Weser 1963; Koch-Weser and Blinks 1963). On the other hand, although it is generally agreed that the rate of intraventricular pressure development increases with frequency (Mitchell, Wallace and Skinner 1963; Covell et al. 1967; Furnival, Linden and Snow 1970), peak intraventricular pressure during isovolumetric contractions is less affected by frequency (Dale 1930; Rosenblueth et al. 1959; Monroe and French 1961; Covell et al. 1967; Andersson, Gennser and Nilsson 1970; Kavalier et al. 1971; Gennser, J6hannsson and Nilsson 1972). Furthermore, the relation between end diastolic pressure and stroke work has been shown to be unaltered by changes in frequency (Mitchell, Wallace and Skinner 1963). The influence of contraction frequency on maximum contractile force of ventricular segments, as measured by strain gauge arch techniques, has also been demonstrated as minimum (Cotten 1953; Sonnenblick, Morrow and Williams 1966).

One possible explanation of the apparent discrepancy between results obtained on excised ventricular preparations and on intact hearts is that the different modes of excitation of the two types of preparations play a role. Whereas in an isolated papillary muscle all cells can be excited within a few msec, excitation of all parts of an intact ventricle requires an appreciable time, up to 50-150 msec (Scher 1962; Rosen et al. 1970). The ventricular wall stress during contraction should be considered a function of the sequence of excitation of individual ventricle cells and the time course of mechanical activity in these cells. The conduction velocity in the ventricle is unaffected by frequency changes or slightly reduced at high rates (Wiggers 1927; Hoffman and Suckling 1954; present study (VII); Rosen et al. 1970). From studies on isolated papillary muscles (cf. above, page 25), it was found that an increased contraction frequency enhances the degree of activation of heart cells and hastens the time

course of contraction. With increasing frequency, the heart cells would therefore tend to contract more and more out of phase with each other, and this increasing asynchrony might oppose the positive inotropic effect of increased frequency that occurs at the cellular level. The present study (V) lends support to this hypothesis, because the two papillary muscles, mechanically arranged in series to simulate a segment of the ventricular wall, showed different frequency response than either would have shown alone. The length changes of the muscles during contraction increased and their force decreased when they were stimulated 25 or 50 msec apart. This force reduction tended to increase with frequency. The apparent discrepancy between the frequency-dependence of isovolumetric pressure in whole ventricles and isometric force of isolated papillary muscles has been further analysed in the human foetal heart (Gennser, Jóhannsson and Nilsson 1972). The results obtained fully support the proposed mechanism.

Influence of muscle length and contraction frequency on human myocardium

The present study showed that the active and resting force-length curves of human foetal papillary muscles are similar to those generally observed in isolated papillary muscles of adults from mammalian species, including man (Sonnenblick, Braunwald, Morrow 1965). The isometric force of human foetal papillary muscles increased with contraction frequency. At 30°C, the optimum force was reached at a frequency of 84 beats per min. It should be pointed out that an increase in temperature shifts the optimum frequency towards higher values, and at 37°C the active force rose with frequency up to 180 beats per min (Gennser, Jóhannsson and Nilsson 1972). Thus, frequency-induced changes in myocardial contractility seem to be as fundamental a property of human myocardium as of other mammalian species. In (VI), it was demonstrated that the intensity and the time course of the active state in human foetal papillary muscle varied with frequency in the same manner as in rabbit papillary muscle. This suggests that the same mechanisms underlie the inotropic change in both species. Both the duration of the action potential in heart cells and the time from stimulus to 50 % decay of the active state decreased with frequency. This is compatible with the hypothesis that membrane depolarization regulates the time course of the release of activator calcium from intracellular stores, whereas the rate of calcium release is proportional to the concentration of calcium at these storing sites before excitation.

Electrophysiological characteristics of human foetal heart cells are similar to those previously demonstrated for hearts of adults from various mammalian species, including man (Trautwein et al. 1962). However, diastolic depolarization in atrial cells indicating latent pacemaker activity seemed to be more prominent in foetal heart cells than previously reported for adult cells. No atrial action potentials with 'double spikes', as have been observed in human atrial preparation from adults (Sleator and de Gubareff 1962; Fabiato and Fabiato 1971), were identified in foetal cells under the experimental conditions used in the present study. But pronounced notches appeared in action potentials recorded from AV-nodal cells during conditions of poor oxygenation. This indicates non-homogeneous excitation of the nodal cells which might be of the same nature as that responsible for the dissociation of two components in adult human atrial cells (Sleator and de Gubareff 1962; Fabiato and Fabiato 1971). The electrophysiological pattern of human foetal heart cells is mainly settled at an embryological period appreciably earlier than studied in the present investigation. This is evidenced by the fact that resting membrane potential levels and action potential characteristics similar to those reported in the present study have been found in human foetal heart cells at a gestational age of 8 to 12 weeks (Gennser and Nilsson 1970; Tuganowski and Cekański 1971).

6 SUMMARY

A release technique utilizing critical damping of the length recording system was used to determine the force-velocity relation and the time course of the active state of rabbit papillary muscles, and also the stiffness of resting and actively contracting preparations. The time course of the active state was experimentally determined as the shortening velocity of the contractile element against a small load as function of time during the contraction cycle, all measurements being referable to the same length of the contractile element. $V_{\max}$ and P_0 of the force-velocity relation of the contractile element (its shortening velocity at zero load and its maximum force-producing capacity, respectively) were found to vary to the same extent with time throughout contraction. Therefore 'velocity-active state curves' could be transformed into the corresponding 'force-active state curves', i.e. the time course of P_0 . The active state had a slow onset and lacked a distinct plateau phase, indicating that the contractile element in heart muscle is not fully activated during an ordinary contraction cycle. When data were collected from the same isotonic myograms after different degrees of active shortening of the contractile element, both the intensity and the duration at the active state decreased with decreasing length.

At any force level, the stiffness of a resting papillary muscle was found to exceed the stiffness of the same muscle during activity. On this basis, it was concluded that the contraction of heart muscle can be represented by a functional model in which the parallel elastic element is arranged in parallel with both the series elastic element and the contractile element. The extrapolated $V_{\max}$ of the force-velocity curve of the contractile element, compensated for the influence of parallel elasticity, increased with muscle length up to lengths where the active force reached its maximum. The duration of the active state was longer, the greater the muscle length.

Changes in contraction frequency affected both the intensity and the time course of the active state. An increased frequency enhanced its total amplitude, and caused a steeper rise and an earlier decline of the active state.

The influence of changes in contraction frequency and muscle length were found to be similar in rabbit papillary muscles and in papillary muscles

from human foetal hearts at midgestation. Resting membrane potential and action potential characteristics of these foetal heart cells were comparable with those described for heart cell of adult mammals. The duration of the cardiac action potential was shorter, the higher the contraction frequency. Changes of initial muscle length did not affect the time course of the action potential.

The present observations on the dynamic parameters of myocardial contraction are discussed in relation to current concepts of excitation-contraction coupling in heart cells, and the implications of the present findings for the function of the intact heart are considered.

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8 REFERENCES

- ABBOTT, B. C. and W. F. H. M. MOMMAERTS, A study of inotropic mechanisms in the papillary muscle preparation. J. gen. Physiol. 1959. 42. 533-551.
- ANDERSSON, K. -E., G. GENNSER and E. NILSSON, Influence of contraction rate, coronary perfusion pressure and diastolic pressure on the mechanical performance of isolated human foetal hearts. Acta physiol. scand. 1970. suppl. 353. 5-18.
- BEELE, G. W. and H. REUTER, The relation between membrane potential, membrane currents and activation of contraction in ventricular myocardial fibres. J. Physiol. (Lond.) 1970. 207. 211-229.
- BLEICHERT, A. and H. REICHEL, Die Hemmung der Erschlaffung beim Herzmuskel des Kalt- und Warmblüters. Pflügers Arch. ges. Physiol. 1962. 276. 242-249.
- BLEICHERT, A. and H. REICHEL, Der Zeitverlauf der Kontraktionsfähigkeit des Muskels nach plötzlicher Entdehnung während elektrisch ausgelöster Kontrakturen. Pflügers Arch. ges. Physiol. 1968. 303. 99-107.
- BLINKS, J. R. and J. KOCH-WESER, Physical factors in the analysis of the actions of drugs on myocardial contractility. Pharmac. Rev. 1963. 15. 531-599.
- BOWDITCH, H. P., Über die Eigenschaften der Reizbarkeit, welche die Muskelfasern des Herzens zeigen. Berichte über die Verhandlungen der Königlich Sächsischen Gesellschaft der Wissenschaften zu Leipzig. 1871. 23. 653-689.
- BRADY, A. J., Time and displacement dependence of cardiac contractility: problems in defining the active state and force-velocity relations. Fedn. Proc. 1965. 24. (Suppl.) 1410-1420.
- BRADY, A. J., Onset of contractility in cardiac muscle. J. Physiol. (Lond.) 1966. 184. 560-580.
- BRADY, A. J., Active state in cardiac muscle. Physiol. Rev. 1968. 48. 570-600.
- BRUTSAERT, D. L., V. A. CLAES and E. H. SONNENBLICK, Effects of abrupt load alterations on force-velocity-length and time relations during isotonic contractions of heart muscle: load clamping. J. Physiol. (Lond.) 1971 a. 216. 319-330.
- BRUTSAERT, D. L., V. A. CLAES and E. H. SONNENBLICK, Velocity of shortening of unloaded heart muscle and the length-tension relation. Circulation Res. 1971 b. 29. 63-75.

- BUCHTHAL, F., E.KAISER and P.ROSENFALCK, The rheology of the cross striated muscle fibre with particular reference to isotonic conditions. Dan. Biol. Medd. (Kbh.) 1951. 21. 1-318.
- COTTEN, M.DeV., Circulatory changes affecting measurement of heart force in situ with strain gauge arches. Am. J. Physiol. 1953. 174. 365-370.
- COVELL, J.W., J.ROSS, R. TAYLOR, E.H.SONNENBLICK and E.BRAUNWALD, Effects of increasing frequency of contraction on the force velocity relation of left ventricle. Cardiovasc. Res. 1967. 1. 2-8.
- DALE, A.S., The relation between amplitude of contraction and rate of rhythm in the mammalian ventricle. (Including interpretation of the apparent indirect action of the vagus on amplitude of ventricular contraction.) J. Physiol. (Lond.) 1930. 70. 455-473.
- DIAMOND, G., J.S.FORRESTER, J.HARGIS, W.W.PARMLEY, R.DANZIG and H.J.C.SWAN, Diastolic pressure-volume relationship in the canine left ventricle. Circulation Res. 1971. 29. 267-275.
- EBASHI, S. and M.ENDO, Calcium ion and muscle contraction. Prog. Biophys. Molec. Biol. 1968. 18. 123-183.
- EDMAN, K.A.P., The relation between sarcomere length and active tension in isolated semitendinosus fibres of the frog. J. Physiol. (Lond.) 1966. 183. 407-417.
- EDMAN, K.A.P., The rising phase of the active state in single skeletal muscle fibres of the frog. Acta physiol.scand. 1970. 79. 167-173.
- EDMAN, K.A.P. and D.W.GRIEVE, The mechanical parameters of the contraction of single muscle fibres of the frog. J. Physiol. (Lond.) 1966. 184. 21-22 P.
- EDMAN, K.A.P., D.W.GRIEVE and E.NILSSON, Studies of the excitation-contraction mechanism in the skeletal muscle and the myocardium. Pflügers Arch.ges. Physiol. 1966. 290. 320-334.
- EDMAN, K.A.P. and A.KIESSLING, The time course of the active state in relation to sarcomere length and movement studied in single muscle fibres of the frog. Acta physiol.scand. 1971. 81. 182-196.
- EDMAN, K.A.P. and E.NILSSON, Time course of the active state in relation to muscle length and movement: a comparative study on skeletal muscle and myocardium. Cardiovascular Res. 1. 1971 (Suppl. 1) 3-10.
- FABIATO, A. and F.FABIATO, The two components of the human atrial action potential. Circulation Res. 1971. 29. 296-305.
- FALSETTI, H.L., R.E.MATES, D.G.GREENE and I.L.BUNNELL, $V_{\max}$ as an index of contractile state in man. Circulation. 1971. 43. 467-479.
- FRANK, O., Zur Dynamik des Herzmuskels. Z. Biol. 1895. 32. 370-437.
- FRY, D.L., D.M.GRIGGS and J.C.GREENFIELD, Myocardial mechanics: tension-velocity-length relationship of heart muscle. Circulation Res. 1964. 14. 73-85.

- FUNG, Y.C., Comparison of different models of the heart muscle. J.Biomechanics. 1971. 4. 289-295.
- FURNIVAL, C.M., R.J.LINDEN and H.M.SNOW, Inotropic changes in the left ventricle; the effect of changes in heart rate, aortic pressure and end-diastolic pressure. J.Physiol. (Lond.) 1970. 211. 359-387.
- GENNSER, G., M.JÖHANSSON and E.NILSSON, The influence of contraction frequency and of a local anaesthetic (Mepivacaine) on human foetal myocardium. Acta physiol.scand. 1972. In press.
- GENNSER, G. and E.NILSSON, Response to adrenaline, acetylcholine and change of contraction frequency in early human foetal hearts. Experientia. 1970. 26. 1105-1107.
- GIBBONS, W.R. and H.A.FOZZARD, Voltage dependence and time dependence of contraction in sheep cardiac purkinje fibres. Circulation Res. 1971. 28. 446-460.
- GORDON, A.M., A.F.HUXLEY and F.J.JULIAN, The variation in isometric tension with sarcomere length in vertebrate muscle fibres. J.Physiol. (Lond.) 1966. 184. 170-192.
- GRIMM, A.F., K.V.KATELE, R.KUBOTA and W.V.WHITEHORN, Relation of sarcomere length and muscle length in resting myocardium. Am.J.Physiol. 1970. 218. 1412-1416.
- GRIMM, A.F. and W.V.WHITEHORN, Myocardial length-tension sarcomere relationships. Am.J.Physiol. 1968. 214. 1378-1387.
- HAWTHORNE, E.H., Instantaneous dimensional changes of the left ventricle in dogs. Circulation Res. 1961. 9. 110-119.
- HILL, A.V., The heat of shortening and the dynamic constants of muscle. Proc.R.Soc.B. 1938. 126. 136-195.
- HILL, A.V., The abrupt transition from rest to activity in muscle. Proc. R.Soc.B. 1949. 136. 399-420.
- HILL, A.V., The transition from rest to full activity in muscle: the velocity of shortening. Proc.R.Soc.B. 1951 a. 138. 329-338.
- HILL, A.V., The earliest manifestation of the mechanical response of striated muscle. Proc.R.Soc.B. 1951 b. 138. 339-348.
- HOFFMAN, B.F. and E.E.SUCKLING, Effect of heart rate on cardiac membrane potentials and the unipolar electrogram. Am.J.Physiol. 1954. 179. 123-130.
- HUXLEY, A.F., Muscle structure and theories of contraction. Prog. Biophys. biophys.Chem. 1957. 7. 255-318.
- HUXLEY, H.E., The mechanism of muscular contraction. Science (N.Y.) 1969. 164. 1356-1366.
- HUXLEY, H.E., Cross-bridge movement and filament overlap. 1971. Biophys.Soc.Abstr. Fifteenth Annual Meeting. 235 a.
- HUXLEY, H.E. and J.HANSON, Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation. Nature (Lond.) 1954. 173. 973-977.

- HUXLEY, A. F. and R. NIEDERGERKE, Structural changes in muscle during contraction: Interference microscopy of living muscle fibres. Nature. (Lond.) 1954. 173. 971-973.
- HUXLEY, A. F. and R. M. SIMMONS, Proposed mechanism of force generation in striated muscle. Nature. (Lond.) 1971. 233. 533-538.
- JEWELL, B. R. and D. R. WILKIE, An analysis of the mechanical components in frog's striated muscle. J. Physiol. (Lond.) 1958. 143. 515-540.
- JEWELL, B. R. and D. R. WILKIE, The mechanical properties of relaxing muscle. J. Physiol. (Lond.) 1960. 152. 30-47.
- JULIAN, F. J., The effect of calcium on the force-velocity relation of briefly glycerinated frog muscle fibres. J. Physiol. (Lond.) 1971. 218. 117-145.
- KATZ, A. M., Contractile proteins of the heart. Physiol. Rev. 1970. 50. 63-158.
- KATZ, A. M., J. P. GOODHART and H. L. GOODHART (1971). Calcium and the cardiac contractile proteins. In HARRIS, P. and L. OPIE, Calcium and the heart, pp 124-134. Academic Press. London and New York.
- KAUFMANN, R., M. J. LAB, R. HENNEKES and H. KRAUSE, Feedback interaction of mechanical and electrical events in the isolated mammalian ventricular myocardium. Pflügers Arch. ges. Physiol. 1971. 324. 100-123.
- KAVALER, F., R. S. HARRIS, R. J. LEE and V. J. FISHER, Frequency-force behavior of in situ ventricular myocardium in the dog. Circulation Res. 1971. 28. 533-544.
- KOCH-WESER, J. and J. R. BLINKS, The influence of the interval between beats on myocardial contractility. Pharmac. Rev. 1963. 15. 601-652.
- KOCH-WESER, J., Effect of rate changes on strength and time course of contraction of papillary muscle. Am. J. Physiol. 1963. 204. 451-457.
- LANGER, G. A., Ion fluxes in cardiac excitation and contraction and their relation to myocardial contractility. Physiol. Rev. 1968. 48. 708-757.
- LEVINE, H. J. and N. A. BRITMAN, Force-velocity relations in the intact dog heart. J. Clin. Invest. 1964. 43. 1383-1396.
- LEWI, P. J., W. K. A. SCHAPER and A. H. M. JAGENEAU, Analysis of the isovolumic pressure in the canine left ventricle. Pflügers Arch. ges. Physiol. 1971. 329. 9-22.
- LEYTON, R. A., H. M. SPOTNITZ and E. H. SONNENBLICK, Cardiac ultrastructure and function: sarcomers in the right ventricle. Am. J. Physiol. 1971. 221. 902-910.
- LITTLE, R. C. and W. B. WEAD, Diastolic viscoelastic properties of active and quiescent cardiac muscle. Am. J. Physiol. 1971. 221. 1120-1125.
- LUNDIN, G., Mechanical properties of cardiac muscle. Acta physiol. scand. 1944. 7. suppl. 20. 7-85.

- MANRING, A. and P. B. HOLLANDER, The interval-strength relationship in mammalian atrium: a calcium exchange model. I. Theory. Biophys. J. 1971. 11. 483-501.
- MITCHELL, J. H., A. G. WALLACE and N. S. SKINNER, Intrinsic effects of heart rate on left ventricular performance. Am. J. Physiol. 1963. 205. 41-48.
- MONROE, R. G. and G. N. FRENCH, Left ventricular pressure-volume relationships and myocardial oxygen consumption in the isolated heart. Circulation Res. 1961. 9. 362-374.
- MORGENSTERN, C., G. ARNOLD, U. HÖLJES and W. LOCHNER, Die Druckanstiegsgeschwindigkeit im linken Ventrikel als Mass für die Kontraktilität unter verschiedenen hämodynamischen Bedingungen. Pflügers Arch. ges. Physiol. 1970. 315. 173-186.
- NOBEL, M. I. M., T. E. BOWEN and L. L. HEFNER, Force-velocity relationship of cat cardiac muscle, studied by isotonic and quick-release techniques. Circulation Res. 1969. 24. 821-833.
- NOBLE, M. I. M., E. N. C. MILNE, R. J. GOERKE, E. CARLSSON, R. J. DOMENECH, K. B. SAUNDERS and J. I. E. HOFFMAN, Left ventricular filling and diastolic pressure-volume relations in the conscious dog. Circulation Res. 1969. 24. 269-283.
- PATTERSON, S. W., H. PIPER and E. H. STARLING, The regulation of the heart beat. J. Physiol. (Lond.) 1914. 48. 465-513.
- PODOLSKY, R. J. and L. E. TEICHHOLZ, The relation between calcium and contraction kinetics in skinned muscle fibres. J. Physiol. (Lond.) 1970. 211. 19-35.
- REICHEL, H. and A. BLEICHERT, Hemmung der Erschlaffung beim Muskel. Pflügers Arch. ges. Physiol. 1962. 275. 526-533.
- RITCHIE, J. M., The duration of the plateau of full activity in frog muscle. J. Physiol. (Lond.) 1954 a. 124. 605-612.
- RITCHIE, J. M., The effect of nitrate on the active state of muscle. J. Physiol. (Lond.) 1954 b. 126. 155-168.
- ROSEN, K. M., S. H. LAU, M. B. WEISS and A. N. DAMATO, The effect of lidocaine on atrioventricular and intraventricular conduction in man. Am. J. Cardiol. 1970. 25. 1-5.
- ROSENBLUETH, A., J. ALANIS, R. RUBIO and E. LOPEZ, The two staircase phenomena. Archs. int. physiol. Biochim. 1959. 67. 374-383.
- RUMBERGER, E., Der Zeitverlauf der Kontraktionsfähigkeit des Herzmuskels nach plötzlichen Entdehnungen während der isometrischen Kontraktion in Abhängigkeit von der Reizfrequenz. Pflügers Arch. ges. Physiol. 1970. 318. 353-365.
- RUMBERGER, E. and B. SCHWARTZ, Das Release-Recovery-Phänomen am Herzmuskel des Frosches bei variierten Ca^{++} -Konzentrationen und Temperaturen. Pflügers Arch. ges. Physiol. 1969. 312. 149-160.
- RUSHMER, R. F., Anatomy and physiology of ventricular function. Physiol. Rev. 1956 a. 36. 400-425.

- RUSHMER, R. F., Initial phase of ventricular systole: asynchronous contraction. Am.J. Physiol. 1956 b. 184. 188-194.
- SANDOW, A., Skeletal muscle. A.Rev. Physiol. 1970. 32. 87-138.
- SANDOW, A., S. R. TAYLOR and H. PREISLER, Role of the action potential in excitation-contraction coupling. Fedn. Proc. 1965. 24. 1116-1123.
- SARNOFF, S. J., Myocardial contractility as described by ventricular function curves; Observations on Starling's law of the heart. Physiol. Rev. 1955. 35. 107-122.
- SARNOFF, S. J. and J. H. MITCHELL, The regulation of the performance of the heart. Am.J. Med. 1961. 30. 747-771.
- SCHAPER, W. K. A., P. LEWI, and A. H. M. JAGENEAU, The determinants of the rate of change of the left ventricular pressure (dp/dt). Arch. für Kreislaufforschung. 1965. 46. 27-41.
- SCHER, A. M. (1962). Excitation of the heart. In HAMILTON, W. F. and DOW, P., Circulation, vol. 1, section 2. American Physiological Society, Washington, D. C.
- SLEATOR, W. and T. DE GUBAREFF, Transmembrane action potentials and contractions of human atrial muscle. Am.J. Physiol. 1964. 206. 1000-1014.
- SONNENBLICK, E. H., Force-velocity relations in mammalian heart muscle. Am.J. Physiol. 1962. 202. 931-939.
- SONNENBLICK, E. H., Active state in heart muscle. Its delayed onset and modification by inotropic agents. J. gen. Physiol. 1967. 50. 661-676.
- SONNENBLICK, E. H., E. BRAUNWALD and A. G. MORROW, The contractile properties of human heart muscle: studies on myocardial mechanics of surgically excised papillary muscles. J. Clin. Invest. 1965. 44. 966-977.
- SONNENBLICK, E. H., A. G. MORROW, and J. F. WILLIAMS, Effects of heart rate on the dynamics of force development in the intact human ventricle. Circulation. 1966. 33. 945-951.
- SONNENBLICK, E. H., W. W. PARMLEY and C. W. URSCHEL, The contractile state of the heart as expressed by force-velocity relations. Am.J. Cardiol. 1969. 23. 488-503.
- SONNENBLICK, E. H., J. ROSS, J. W. COVELL, H. M. SPOTNITZ and D. SPIRO, The ultrastructure of the heart in systole and diastole. Changes in sarcomere length. Circulation Res. 1967. 21. 423-431.
- SONNENBLICK, E. H. and A. C. STAM, Cardiac muscle: activation and contraction. A.Rev. Physiol. 1969. 31. 647-674.
- SPOTNITZ, H. M., E. H. SONNENBLICK and D. SPIRO, Relation of ultrastructure to function in the heart: sarcomere structure relative to pressure volume curves of intact left ventricles of dog and cat. Circulation Res. 1966. 18. 49-66.

- TAYLOR, C. P. S., Isometric muscle contraction and the active state: an analog computer study. Biophys. J. 1969. 9. 759-780.
- TAYLOR, R. R., J. ROSS, J. W. COVELL and E. H. SONNENBLICK, A quantitative analysis of left ventricular myocardial function in the intact, sedated dog. Circulation Res. 1967. 21. 99-115.
- TAYLOR, S. R. and R. RÜDEL, Striated muscle fibers: inactivation of contraction induced by shortening. Science (N. Y.) 1970. 167. 882-884.
- TRAUTWEIN, W. and J. DUDEL, Aktionspotential und Mechanogramm des Warmblüterherzmuskels als Funktion der Schlagfrequenz. Pflügers Arch. ges. Physiol. 1954 a. 260. 24-39.
- TRAUTWEIN, W. and J. DUDEL, Aktionspotential und Mechanogramm des Katzenpapillarmuskels als Funktion der Temperatur. Pflügers Arch. ges. Physiol. 1954 b. 260. 104-115.
- TRAUTWEIN, W., D. G. KASSEBAUM, R. M. NELSON, and H. H. HECHT, Electrophysiological study of human heart muscle. Circulation Res. 1962. 10. 306-312.
- TUGANOWSKI, W. and A. CEKAŃSKI, Electrical activity of a single fibre of the human embryonic heart. Pflügers Arch. ges. Physiol. 1971. 323. 21-26.
- WALLACE, A. G., N. S. SKINNER and J. H. MITCHELL, Hemodynamic determinants of the maximal rate of rise of left ventricular pressure. Am. J. Physiol. 1963. 205. 30-36.
- WEAD, W. B. and R. C. LITTLE, Effect of stress relaxation on the contraction response of cardiac muscle. Proc. Soc. Biol. Med. 1971. 137. 939-941.
- WIGGERS, C. J., Are ventricular conduction changes of importance in the dynamics of ventricular contraction? Am. J. Physiol. 1927. 80. 12-30.
- WISE, R. M., J. F. RONDINONE and F. N. BRIGGS, Effect of calcium on force-velocity characteristics of glycerinated skeletal muscle. Am. J. Physiol. 1971. 221. 973-979.
- WOOD, E. H., R. L. HEPPNER and S. WEIDMANN, Inotropic effects of electric currents. I. Positive and negative effects of constant electric currents or current impulses applied during cardiac action potentials. II. Hypotheses: calcium movements, excitation-contraction coupling and inotropic effects. Circulation Res. 1969. 24. 409-445.
- WOODWORTH, R. S., Maximal contraction, "staircase" contraction, refractory period, and compensatory pause, of the heart. Am. J. Physiol. 1903. 8. 213-249.

